Ecological Indicators 101 (2019) 533–540

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Predicting thermal sensitivity of three Amazon fishes exposed to climate change scenarios

D.F. Campos*, S. Braz-Mota, A.L. Val, V.M.F. Almeida-Val

LEEM – Laboratory of Ecophysiology and Molecular Evolution, Brazilian National Institute for Research of the Amazon, Manaus, Amazonas, Brazil

ARTICLE INFO

Keywords:
Metabolic stress
Antioxidant capacities
Thermal tolerance
Acclimation capacities
Integrated biomarkers response

ABSTRACT

Increased temperature and CO_2 levels are predicts for the next decades. Tropical species are expected to be especially vulnerable to these alterations once many of them appear to have a narrower thermal tolerance range compared to subtropical and temperate species; and live closer to their thermal limits. Herein, we experimentally investigated the effects of climate change scenario on metabolic and oxidative stress in three ornamental fishes of Amazon forest streams. *Apistogramma agassizii, Pyrhullina brevis* and *Hyphessobrychon melazonatus* were exposed to current and extreme climate scenarios (4.5 °C and 900 ppm CO_2 above current levels) and had respiratory profile, antioxidant enzymes and neurotransmitter responses evaluated. The integrated biomarkers response index (IBR) was calculated to examine specie's acclimation abilities. After 30 days of exposure, we observed distinct physiological mechanisms to cope with climate change. Overall, Amazonian fish species are susceptible to climate change since they showed increase in metabolic rate and oxidative stress. Yet, sedentary ones *A. agassizii* and *P. brevis*, appeared to be less impacted by climate change than *H. melazonatus*, once they presented high survival rates, thermal tolerance and low IBR values. In contrast, *H. melazonatus*, an athletic species, low survival rates, lipid peroxidation, lower thermal tolerance and high IBR. This study provides evidence that future climate changes will affect energy supply and promote species-specific damages in metabolic pathways, with consequent physiological impairments, which may have detrimental effects at population and ecosystem levels.

1. Introduction

Several models of climate changes predicts an increase in tropical water surface temperatures up to 3 °C for the next century (Lough, 2007; Meehl et al., 2007; Ganachaud et al., 2011; IPCC, 2014). Some models predict a temperature increase of 3–5 °C during this period for the Amazon region (Marengo, 2009, PBMC, 2013). Besides climate change, riparian deforestation is increasing in the Amazon region and has increase temperature of small streams up to 5 °C (Macedo et al., 2013). Temperature is one of the most important environmental factors affecting the performance and distribution of organisms (Madeira et al., 2016a). However, little information exists concerning the Amazon stream fish physiology responses to changes in temperature.

Temperature has profound impacts on chemical and biochemical reactions, influencing the whole ectothermic aerobic metabolism. Accordingly, warming increases endogenous Reactive Oxygen Species (ROS) in ectothermic organisms, with potential impacts to energy supply and performance (Madeira et al., 2012). Moreover, atmospheric CO_2 levels are expected to reach values up to 940 ppm by the end of the twenty-first century (IPCC, 2014). CO_2 reacts with water resulting in an increase of H^+ and HCO_3^- concentrations and lower CO_3^{2-} levels. This process, named water acidification is expected to acidify the surface water by 0.14–0.42 units by the end of this century (Pörtner et al., 2014). Physiologically, water acidification disrupts the acid-base balance of fish, with consequences to metabolic performance (Rosa et al., 2016). Also, elevated CO_2 concentrations induce oxidative stress due to the parallel increase of H^+ ions (extra and intracellular acidosis) (Feder and Hofmann, 1999; Dean, 2010; Sampaio et al., 2018).

Tropical species are expected to be especially vulnerable to warming because many of them appear to have a narrower thermal range compared to subtropical and temperate species, and tend to live closer to their thermal limits (Pörtner and Farrell, 2008). Therefore, even small increases could lead to declines in individual and populations performance (Stillman, 2003; Tewksbury et al., 2008). Actually, Campos et al. (2018) showed that active species have lower thermal tolerance compared to sedentary ones when analysing fish inhabiting forest streams of the Amazon; however, their acclimation abilities has not been studied yet. Besides, Campos et al. (2017) studying palm

* Corresponding author.
E-mail address: campos.derek@gmail.com (D.F. Campos).

https://doi.org/10.1016/j.ecolind.2019.01.051
Received 12 September 2018; Received in revised form 21 January 2019; Accepted 22 January 2019
Available online 29 January 2019
1470-160X/ © 2019 Elsevier Ltd. All rights reserved.

swamp species showed that the congeneric species, *Paracheiron axelrodi* and *P. simulans*, have low acclimation abilities and decreased thermal safety margin at high acclimation temperature. Species vulnerability to warming has been associated with loss of aerobic capacity and oxidative damage, in particular, to the constraints of decreased oxygen supply and increased demand. According to the OCLTT (oxygen- and capacity limited thermal tolerance) hypothesis, the aerobic capacity is limited by insufficient oxygen supply and sets the performance in animals (e.g. Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner et al., 2010).

The ability of fish to respond to environmental stress depends on the management of internal components and processes to maintain physiological homeostasis (Somero, 2010). Mechanisms involving physiological homeostasis are mainly related to oxidative defense capacity and the mechanisms of alterations of general energetic metabolism (Farrell, 2007; Pörtner and Farrell, 2008; Somero, 2010; Madeira et al., 2016a). Warming and high pCO_2 induces cellular redox changes and have been reported to activate reactive oxygen species (ROS) scavengers (such as catalase, glutathione-S-transferase and peroxidases) to reduce the flux of ROS produced during oxidative metabolism (Somero, 2010). However, when the production of ROS exceeds levels of antioxidant defense, the oxidative stress occurs (Vinagre et al., 2012). Overproduction of ROS can damage important biomolecules, such as DNA, proteins, and lipids, and initiate a cascade of events, bringing about impaired cellular function and even apoptosis events (Iftikar and Hickey, 2013). Rosa et al. (2016) reported a substantial vulnerability of tropical shark (*Chiloscyllium punctatum*) exposure to climate change scenario (+4 °C and pH 7.5) related to disruption of the brain neurotransmitter, increases in oxidative stress and consequent decrease in aerobic capacities. However, some species demonstrate physiological mechanisms that allow overcoming the negative consequences of thermal stress, which includes the avoidance of free radicals accumulation, protein degradation and energy depletion (Hofmann and Somero, 1995; Tomanek, 2010). For instance, Lopes et al. (2018) suggest that ROS-scavenging molecules provide an effective defense mechanism in dealing with ROS formation in newly hatched sharks (*Chiloscyllium plagiosum*) allowing this species overcoming the impacts of upcoming climate change scenario.

The Amazon forest streams are a thermo-stable environment that support high fish diversity with a variety of lifestyles. The species have important ecological roles, such as energy cycling and transfer between land and aquatic environments. In addition, fish species from Amazon forest stream have an important economic role since most of them are used in ornamental trades. Even though they are not considered at risk by conservational programs, climate change can affect their survival. Therefore, understanding the acclimation abilities of these species are essential to comprehend the climate change effects and use these information for conservation programs.

In this sense, the capacity of species to deal with free radicals and to maintain aerobic capacity and thermal safety margin can be a critical point for defining their vulnerabilities to near-future climate change scenarios (Deschaseaux et al., 2010). These biological responses can be used as stress biomarkers providing early warning signals of physiological stress, and could indicate occurrences of adverse ecological consequences in fish communities (Wu et al., 2005). Therefore, in this study, we experimentally investigated the effects of two climate change scenarios on metabolic and oxidative stress in three Amazon forest stream fishes. In particular, the main goals were: (i) to investigate the effects of two climate scenarios on the metabolic profile of three species; (ii) to perform a comparative assessment of extreme climate change scenario at a molecular biomarker of oxidative effects in white muscle tissue; and (iii) to analyze the acclimation capacities and thermal limits in which the physiological responses are modulated by climate change scenarios. We hypothesize that exposure to extreme climate change scenario (4.5 °C and 900 ppm CO2 higher than current scenario) Amazon forest stream fishes will increase metabolic demand and oxidative stress indicating low acclimation abilities.

2. Material and methods

2.1. Collection and maintenance of fish

We selected three species based on their distinct life-styles and swimming behavior, which according to our previous work (Campos et al., 2018), can be identified by their physiological abilities to deal with warming environment: *Apistogramma agassizii*, *Pyrrhullina brevis* and *Hyphessobrychon melazonatus*. Fishes were collected in first- and second-order streams at Reserva Ducke (02°53′S, 59°58′W), a protected area located in the central Brazilian Amazon near the confluence of the Negro and Solimões rivers, bordering Manaus. Fish were collected by hand and seine nets (2-mm mesh) (see Mendonça et al., 2005 for details). Captured fish were maintained in a 50-L aquarium with constant aeration and transferred to LEEM – Laboratory of Ecophysiology and Molecular Evolution at Brazilian National Institute for Research of the Amazon – INPA. Each species was held at one 150-L tanks (Fortlev®) for two-weeks before experiments. Half of the water was replaced every 24 h. During this period, fish were fed once a day until satiation with TetraMin® Flakes. The temperature was maintained close to that of the streams (26 ± 1.0 °C). All housing and experimental procedures were in accordance with CONCEA Brazilian Guide for Animal Use and Care and were authorized by INPA's Council for Ethics in Animal Use (CEUA – protocol number 027/2015).

2.2. Climate scenarios exposure

The three species (*A. agassizii*, *P. brevis* and *H. melazonatus*) were exposed to two distinct climate scenarios at controlled environmental rooms. This system consists of environmental rooms with temperature and CO_2 controlled by computer according to different scenarios. The climate scenarios provided by the Assessment Report of the IPCC (2014) for the year 2100 were simulated in these environmental rooms: the current scenario (current temperature and CO_2 levels) that was set according to data acquisition every two minutes at the natural forest by Fieldlogger 512 k (Novus Produtos eletrônicos LTDA); and A2 room (extreme scenario) that was set at 4.5 °C and 900 ppm CO_2 above the current scenario. The sensors at natural forest measured temperature and CO_2 every two minutes and transmitted the data to the laboratory computers that control the environmental rooms according to the above scenarios in real time. Data of room's temperature and CO_2 are available in Supplementary material (SM1).

Adults of the three chosen species were transferred to 10-L PVC tanks in three replicates per species containing six individual each tank, in both scenarios, N = 36 per species. They were maintained in each climate room for 30 days, during November 2017 (Amazon dry season). To avoid ammonia accumulation, 30% of the water was replaced every day using environmentally stabilized water. The pH, O_2, CO_2 and temperature levels of the water were daily measured (Table 1). The fish were fed *ad libitum* once a day using commercial TetraMin®Flakes and fish were unfed 48 h before experiments.

2.3. Biomarkers assays

2.3.1. Oxygen consumption

After 30 days acclimation we measured fish oxygen consumption in a closed chamber system. For that purpose, individuals were transferred to a 150 mL PVC chamber for *A. agassizii* and *H. melazonatus* and 500 mL PVC chamber for *P. brevis*, maintaining the same proportion of water volume/fish weight. We sampled 8 individuals for *A. agassizii* and *P. brevis*, 3 individuals from two aquariums and 2 from another aquarium in each climate scenario, and for *H. melazonatus* we sampled 2 individuals from each aquarium in each climate scenario. Fish were allowed to handily recover for 2 h in fully oxygenated chamber. After

Table 1
Temperature, O_2, CO_2, and pH means in the water and in the air of experimental climate rooms; The rooms are real-time computer controlled simulating current levels and extreme climate scenarios (A2, plus 4.5 °C and 900 ppm CO_2) proposed by IPCC 2014.

	Water				Air	
Scenario	Temperature °C	O_2 (mg·L^{-1})	CO_2 (ppm)	pH	Temperature °C	CO_2 (ppm)
Current	26.5 ± 1.0	6.5 ± 0.5	11 ± 2	6.4 ± 0.3	27.5 ± 1.7	476 ± 15
Extreme	30.5 ± 1.0	6.3 ± 0.4	28 ± 2	5.8 ± 0.5	32.0 ± 1.8	1294 ± 16

this period, the oxygen of the chamber was shut-off, and the fish oxygen consumption was monitored by 15 min using an Oxy-4 oximeter (Loligo system). After this period, the oxygen was turned-on for 45 min. Also, background respiration (chamber without fish) was measured concomitantly. The oxygen consumption was measured three times for each individual during 4 h, so we calculated the mean of the replicate of oxygen consumption following: MO_2 ($\mu LO_2 \cdot h^{-1} \cdot kg$) = $-\Delta O \cdot Vresp \cdot B^{-1}$, where ΔO is the rate of change in oxygen concentration ($\mu LO_2 \cdot h^{-1}$), Vresp is the volume of the respirometric chamber, and B is the mass of the individual (kg). After that, fishes were anesthetized by concussion and euthanized by medullar section. Muscle was removed, immersed in liquid N_2 and stored in an ultrafreezer at −80 °C until enzyme assays.

2.3.2. Metabolic enzymes

For metabolic enzyme assays, lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and citrate synthase (CS), 0.01 g of white muscle was individually homogenized in a imidazole buffer (150 mM imidazole, 1 mM EDTA and TritonX at pH 7.4) followed by centrifugation at 10,000*g* in a Sorvall RC-5B for 15 min at 4 °C. The activities of LDH and MDH were measured following the oxidation of NADH at 340 nm and the oxidation of DTNB (5,5′-Dithiobis(2-nitrobenzoic acid) at 412 nm for CS (for details see Driedzic and Almeida-Val (1996), modified by Campos et al., 2017). The enzyme activities were determined at 25 °C using a SpectramaxPlus 384 (Molecular Device, USA). The protein was quantified by Bradford assay (Bradford, 1976).

The oxidative phosphorylation capacity was measured by electron transport system (ETS) activities. ETS was measured using the method proposed by Packard (1971) and improved by G.-Tóth (1993). The ETS activity of each animal was determined using the procedure described in Simčič and Brancelj (2003, for detail see). ETS activity was measured as the rate of tetrazolium reduction to formazan and converted to equivalent oxygen consumed per mg of wet mass per hour ($\mu LO_2 \cdot mg^{-1} \cdot h^{-1}$) as described by Kenner and Ahmed (1975).

2.3.3. Antioxidant enzymes and LPO levels

The glutation-S-transferase (GST) activity in white muscle was determined using 1-chloro 2,4-dinitrobenzene (CDNB) as substrate, according to Keen et al. (1976). Changes in absorbance were recorded at 340 nm. The enzyme activity was calculated as nmol CDNB conjugate formed per min per mg protein. To determine the catalase (CAT) activity, the inhibition rate of H_2O_2 decomposition was monitored at 240 nm (Beutler, 1975), and expressed as μM H_2O_2 min^{-1} mg $protein^{-1}$. The lipid peroxidation (LPO) levels were quantified based on the oxidation of the Fe^{+2} to Fe^{+3} by hydroperoxides in acid medium in the presence of ferrous oxidation-xylenol orange, at 560 nm, according to the method described by Jiang et al. (1991).

2.3.4. Acetylcholinesterase activity (AChE)

The AChE activity in white muscle was determined by the method of Ellman et al. (1961). Briefly, samples were homogenized in phosphate buffer 1:4 (0.1 M, glycerol 20%, pH 7.5), centrifuged for 20 min at 12,000*g* (4 °C) and the supernatant used for measurement of AChE activity. Acetylthiocholine iodide (ATC) 9 mM was used as a substrate and 5,50-dithio-bis (2-nitrobenzoic) acid (DTNB) as color reagent. Kinetic activity of AChE was measured at 415 nm and the units are μM min^{-1}·mg $protein^{-1}$.

2.4. IBR calculations

The integrated biomarkers response index (IBR) was calculated according to Beliaeff and Burgeot (2002), as the triangular star plot areas calculated for each two neighboring data. To calculate IBR, the general mean (*m*) and the standard deviation (*s*) of all data regarding a given biomarker was calculated, followed by a standardization to obtain *Y*, where $Y = (X - m)/s$, and *X* is the mean value for the biomarker at a given temperature. Then, *Z* was calculated using $Z = -Y$ or $Z = Y$, in the case of a biological effect corresponding respectively to an inhibition or a stimulation. Afterwards, the score (*S*) was calculated by $S = Z + |Min|$, where $S \geq 0$ and $|Min|$ is the absolute value for the minimum value for all calculated *Y* in a given biomarker at all measurements made. Star plots were then used to display Score results (*S*) and to calculate the integrated biomarker response (IBR) as:

$$IBR = \sum_{t=1}^{n} A_i$$

$$A_i = \frac{S_i \times S_{i+1} \times sina}{2}$$

where *Si* and *Si + 1* are two consecutive clockwise scores (radius coordinates) of a given star plot; *Ai* corresponds to the area of the connecting two scores; and $\alpha = 2\pi/n$ where *n* is the number of biomarkers used for calculations. The IBR is obtained by summing up all the *Ai*. The IBR calculations were always performed with the same order of biomarkers for all temperatures taking into account their biological function (MO_2, ETS, CS, MDH, LDH, CAT, GST, LPO, Ache).

2.5. Critical thermal tolerance

Thermal limits were estimated using critical thermal methodology (CTM) (Lutterschmidt and Hutchison, 1997). After the acclimation period, in each climate change scenario, five individuals of each species were held in 10 L aquaria with a digital thermostat to control temperature (TIC-17RGT, FullGauge, ± 0.01 accuracy) and allowed handily recover for 24 h. After this period, the thermal ramp was initiated with an increase of 0.3 °C/min according to a review of Lutterschmidt and Hutchison, 1997. The critical thermal maximum was defined as the temperature at the fish presented a final loss of equilibrium, or LOE (inability to maintain dorsal-ventral orientation for at least 1 min, Beitinger and Bennett, 2000).

2.6. Statistical analyses

The data are presented as mean ± SD (n = 8–6). All statistical analyses were carried out in SigmaStat 3.1 using a significance level of 5%. Survival rate was measured along the experiment and tested using Using Kaplan-Meier analysis among species. Parametric Test t or non-parametric Mann-Whitney (depending on whether the data met the assumptions of normality and homoscedasticity) were performed to detect significant differences in the biomarkers end-points and CTMax among different climate change scenarios within each species. Also, we carried out cluster analyses and heatmaps in MetaboAnalyst 4.0 (R package; according to Chong et al., 2018) using glog transformed and auto-scaled data, Cluster analyses followed the criteria (i) cluster rows,

(ii) similarity metrics: Euclidean, iii) clustering method: Ward. The colour scale in heatmaps ranges from red (higher than mean biomarker concentration/activity) to blue (lower than mean biomarker concentration/activity).

3. Results

Significant differences in abiotic factors measured between climate change scenarios were observed. The extreme climate scenario presented higher temperature and CO_2 levels in the air, and these parameters followed in the water where, in the A2 climate room, water temperature, CO_2 were higher and pH was lower (mean of 30.5 °C, 27 ppm CO_2, and 5.8 pH) compared to the current scenario (mean of 26.5 °C, 11 ppm CO_2, and 6.5 pH) (Table 1). The CO_2 reacts with water resulting in an increase of H^+ and HCO_3^- concentrations and lower CO_3^{2-} levels, therefore significant part of the CO_2 was responsible by the decrease of pH at the extreme scenario (Table 1). It is important to mention that, in natural systems, groundwater could alter the pattern we have observed here.

The survival rate at A2 scenario differed among the three species ($p > 0.001$); *P. brevis* presented 83% of survival along the experiment, while *H. melazonatus* showed 66% of survival rate. We did not observe mortality in the *A. agassizii* along the experiment (Fig. 1). Results of statistical analyses showed distinct adaptation mechanisms to face the climate change scenarios among the studied species (Table 2; Fig. 4). Oxygen consumption was the single parameter that increased in all species (Fig. 2).

Appistogramma agassizii increased LDH and decreased ETS activities, indicating metabolic depression and an activation of the anaerobic pathway in white muscle (Table 2). In addition, the antioxidant enzyme GST and LPO increased. Also, this species presented an increased activity of the enzyme AChE (Table 2). Although *A. agassizii* increased oxidative stress, the IBR increased by 1.7 times, indicating a high capacity to acclimate to the extreme scenario (Fig. 3). This is well supported by 1.5 °C increase in thermal tolerance to the scenario A2 (current scenario 40 °C and A2 41.5 °C; Fig. 5).

Besides increased oxygen consumption, *P. brevis* increased CAT and AChE activities (Table 2), and increased IBR by 1.9 times at extreme scenario compared to the current scenario (Fig. 3). In addition, this species increased critical thermal maximum by 1 °C in average in thermal tolerance to the extreme scenario (current 39 °C and A2 40 °C; Fig. 5).

In contrast, *H. melazonatus* presented the higher alterations in endpoint biomarkers. This species increased the Krebs' cycle oxidation, indicated by citrate synthase activity and also by the activation of antioxidant defense, observed by the increase in the CAT activity, with a consequent increase in LPO, indicating high levels of oxidative damage (Table 2). Interestingly, this species decreased AChE activity, an opposite trend when compared to the others species. Moreover, *H. melazonatus* presented the highest increase in IBR, i.e., 2.58 times (Fig. 3) and did not change thermal tolerance (36 °C; Fig. 5).

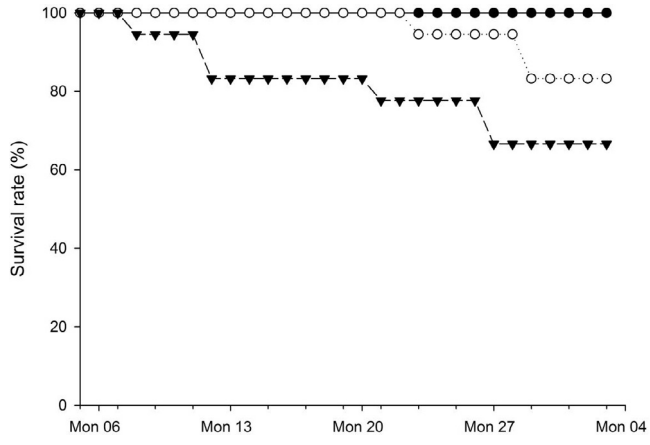

Fig. 1. Survival rate of *Apistogramma agassizi* (—●—), *Pyrrhullina brevis* (···○···), *Hyphessobrychon melazonatus* (–▼–) exposed to warming climate change scenario (A2, 4.5 °C and 900 ppm CO_2 higher than current scenario) proposed by IPCC, 2014. Using Kaplan-Meieranalysis, we observed differences in survival rate ($p > 0.001$).

The cluster analyses and heatmaps indicate a clear segregation between current and extreme scenario. Exposed to extreme climate change scenario (A2; IPCC, 2014) all species presented an increase in biomarkers levels. In addition, *H. melazonatus* present the large biomarkers increase under climate change scenario (Fig. 4). All these data indicate low acclimation ability and high vulnerability to climate change scenario for this specie.

4. Discussion

Warming and high pCO_2 levels have negative impacts on ectotherms increasing metabolic demands to deal with physiological stress. The temperature increase affects the rate of reactions and promotes cellular damage, while high pCO_2 increases the energy consuming for ion and acid–base regulatory mechanisms. Thus, the energetic costs associated with maintaining homeostasis in situations of chronic exposure to climate change can alter the finite energy budgets resulting in a reduction in growth, reproduction and survival performance (Pörtner and Knust, 2007; Madeira et al., 2016a,b; Rosa et al., 2016; Ilha et al., 2018).

Predictions of climate change impacts on populations and ecosystems require the assessment of physiological capacity to acclimation and tolerance (Semsar-kazerouni and Verberk, 2018). The findings of this study suggest that active life-style fish, such as *H. melazonayus*, lacks the ability to tolerate or acclimate to a new climate scenario, as opposed to the sedentary ones, *P. brevis* and *A. agassizi*. This outcome is supported by the high mortality rates, increased oxidative stress and loss of neurotransmission capacity endured by *H. melazonatus* at A2 (*sensu* IPCC, 2014) climate scenario. This result confirms that near-future climate scenario will have lower impact on fish species with low activity performances, in accordance with former studies (Stoffels, 2015; Campos et al., 2018). These findings imply that athletic fishes may have less capacity to survive under climate change scenario due to a low acclimation capacity. In contrast, sedentary species may have physiological traits that increase resistance and facilitate local persistence. The energetic cost for activity in fast species, combined with energetic cost of homeostasis should decrease the energy amount to deal with further energetic demand at warming, overwhelming their energy capacity.

4.1. Biomarkers response to climate change exposure

Acclimation capacities to warming and hypercapnia have been related to aerobic compensation that improves tolerance and maintains homeostasis. In the present work, a consistent result with previous studies (Peck et al., 2012; reviewed by Schulte, 2015). For Amazon species, Campos et al. (2017) also found that two congeneric ornamental fishes increased their routine metabolic rates at warming acclimation. In a recent review, Peck et al. (2012) found that a 10 °C increase in temperature was accompanied by a 1.2- to 4.3-fold increase in oxygen consumption ($Q10 = 1.2–4.3$) across 15 families of marine fish. Increased energetic costs under chronic exposure to warming can decrease the energy budgets resulting in a reduction in growth, reproduction and survival performance (Pörtner and Knust, 2007; Madeira et al., 2016a,b; Rosa et al., 2016). In fact, Ilha et al. (2018) observed that deforestation causes stream warming and decrease body size of Amazonian fishes.

Although all species increased oxygen consumption, such increase was not related to oxygen demands in the muscle. For instance, *A. agassizii* activated its anaerobic power (LDH activities) and decreased

Table 2
Lactate dehydrogenase (LDH) activity (uMol·min^{-1}·mg^{-1} protein), Malate dehydrogenase activity (MDH) (μMol·min^{-1}·mg^{-1} protein), Citrate synthase activity (CS) (μMol·min^{-1}·mg^{-1} protein), Electron transport system activity (ETS) (μLO2 mg^{-1} h^{-1}), glutathione-S-transferase activity (GST) (μMol·min^{-1}·mg^{-1} protein), Catalase activity (CAT) (μMol H_2O_2·min^{-1}·mg^{-1} protein), Levels of lipoperoxidaton (LPO) (μMol cumeme hydroperoxide mg. protein) and Acetilcolinestarase activity (Ache) (μMol·min^{-1}·mg^{-1} protein) measured in white muscle of *Appistogramma agassizii*, *Pyrrhullina brevis* and *Hyphessobrychon melazonatus* exposed to current scenario and extreme climate change scenario (A2; 4.5 °C and 900 ppm CO_2 above current levels). * indicates significant different from current scenario using *t*-test ($p < 0.05$).

		LDH	MDH	CS	ETS	GST	CAT	LPO	Ache
A. agassizii	Current	1.17 ± 0.3	1.33 ± 0.54	0.08 ± 0.04	10,112.9 ± 2070.0	22.52 ± 5.7	0.029 ± 0.02	15.2 ± 6.9	8.12 ± 1.97
	Extreme	1.98 ± 0.7*	1.05 ± 0.34	0.08 ± 0.03	7130.0 ± 980.2*	45.40 ± 9.9*	0.026 ± 0.01	23.8 ± 5.4*	12.14 ± 4.52*
P. brevis	Current	1.51 ± 0.2	1.15 ± 0.24	0.09 ± 0.02	5215.2 ± 1888.2	56.98 ± 14.3	0.005 ± 0.003	16.38 ± 4.3	7.76 ± 1.44
	Extreme	1.72 ± 0.4	1.17 ± 0.23	0.10 ± 0.01	5389.0 ± 1340.0	60.25 ± 21.1	0.015 ± 0.008*	16.05 ± 5.9	11.75 ± 3.65*
H. melazonatus	Current	1.38 ± 0.4	1.04 ± 0.24	0.10 ± 0.01	8489.5 ± 3527.9	50.75 ± 15.0	0.005 ± 0.002	13.72 ± 1.6	7.57 ± 1.26
	Extreme	1.67 ± 0.4	1.24 ± 0.34	0.15 ± 0.03*	7736.4 ± 2099.0	50.70 ± 12.1	0.010 ± 0.004*	21.33 ± 3.8*	5.00 ± 1.20*

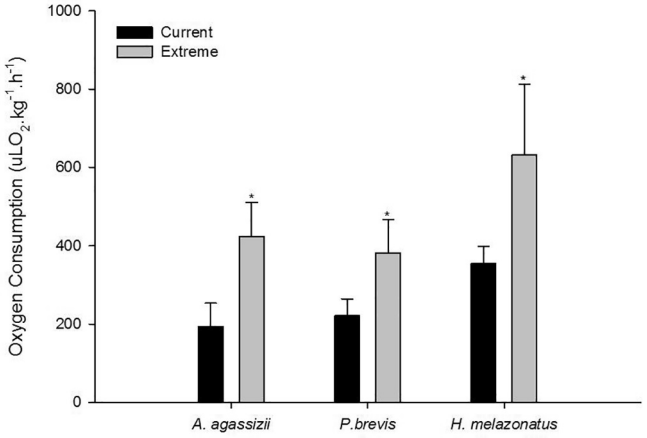

Fig. 2. Oxygen consumption of three Amazon forest stream fishes (*Appistogramma agassizii*, *Pyrrhullina brevis* and *Hyphessobrychon melazonatus)* acclimated for 30 days at climate change scenarios (IPCC 2014). * indicates significant different from current scenario using *t*-test ($p < 0.05$).

oxidative phosphorylation (ETS activities), while *H. melazonatus* increased aerobic oxidation (CS). These species-divergent responses seem to be strongly related to fishes' life-style and swimming behavior. For instance, *A. agassizii* is a sedentary species living in pounds and pools, swimming throughout burst propulsion, which is related to anaerobic power. Therefore, decreasing oxidative phosphorylation does not represent an energetic impairment of movement for this species, once it appears that this species increases its glycolytic power to supply muscle energy. Instead, *H. melazonatus* increased substrate oxidation in the Krebs cycle to support the increase of energy demand. Moreover, this species presented no increase in oxidative phosphorylation (ETS activity), what could mean impairment of electron transport chain, leading to important energy impacts and compromising its swimming performance.

Respiration represents a delicate trade-off, once cellular oxygen increase is both an essential component for aerobic energy generation as well as a toxic component, forming oxygen radicals in the mitochondria in an oxygen-dependent manner. Thus, increasing oxygen consumption to meet energy supply may have an odd cellular cost (Madeira et al., 2016a,b). In the present work, all species increased antioxidant systems in extreme climate change scenario, *A. agassizii* increased GST, while *P. brevis* and *H. melazonatus* increased CAT. These results suggest that an inherent thermal sensitivity of the cell lead to sufficient thermal damage that required the activation of protective processes through activation of ROS scavengers (antioxidants) (Jesus et al., 2017; Sampaio et al., 2018). These results indicate a response to increased oxygen consumption demand, which leads to accelerated mitochondrial respiration uncoupled to ATP generation and, consequently, the generation of oxygen free radicals within tissues caused by warming and high CO_2 levels (Pannunzio and Storey, 1998; Abele et al., 2002). These types of molecular responses have also been observed in previous studies (Madeira et al., 2012; Madeira et al., 2016b; Rosa et al., 2016).

Oxidative stress occurs from the inability of the antioxidant defense system to avoid damage caused by ROS and, although antioxidant enzymes (GST and CAT) acted to detoxify ROS under thermal stress levels, this significant increase was not enough to minimize the drastic increase in peroxidative damage in *A. agassizii* and *H. melazonatus*. The rise of lipid peroxidation levels means cell membrane disruption due to high cellular ROS, which could be related to malfunction of the mitochondrial complex enzymes, leading to a less efficiency of the oxidative phosphorylation and reduced ATP generation. Accordingly, energetic depression is observed as lipid peroxidation rate increases (Abele et al., 2002; Rosa et al., 2016). In fact, these results are corroborated by the energy depletion in *A. agassizii* and *H. melazonatus*. Therefore, thermal and acidification stress might be related to

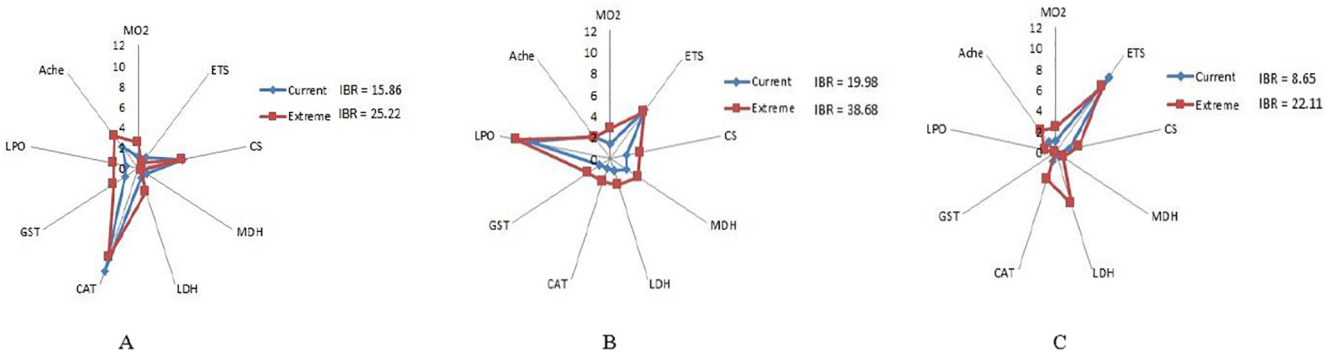

Fig. 3. Integrated biomarker response index (IBR) for muscle of (a) *Apistogramma agassizii* (b) *Pyrrhullina brevis* and (c) *Hyphessobrychon melazonatus* acclimated to current and warming climate scenario (A2; IPCC, 2014).

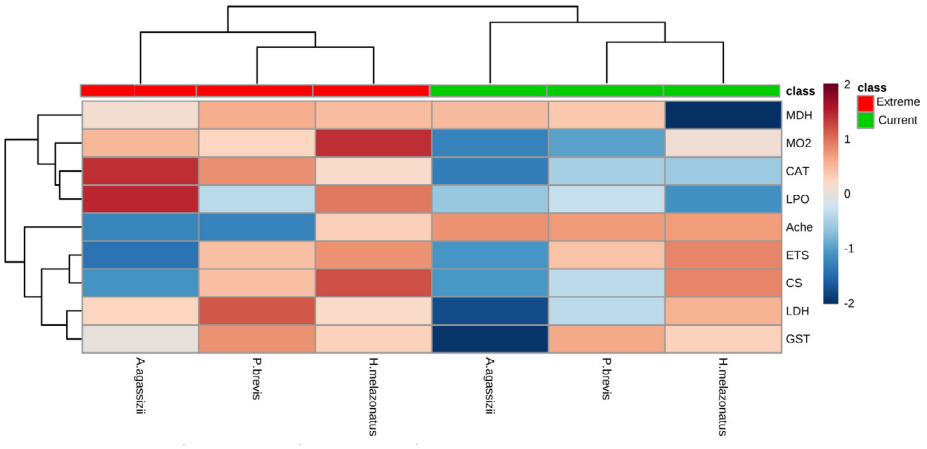

Fig. 4. Heatmap representation of the clustered data of three Amazon forest stream fishes (*Appistogramma agassizii*, *Pyrrhullina brevis* and *Hyphessobrychon melazonatus)* exposed current climate change scenario and extreme (A2, 4.5 °C and 900 ppm CO_2 higher from current scenario). Each cells denote the glog (generalized logarithm transformation) values of biomarker normalized concentration/activity. The colour scale ranges from red (higher than mean biomarker concentration/activity) to blue (lower than mean biomarker concentration/activity). Oxygen consumption (MO_2), Lactate dehydrogenase (LDH), Malate dehydrogenase (MDH, Citrate synthase (CS), Electron transport system (ETS), glutathione-S-transferase (GST, Catalase activity (CAT), lipoperoxidaton (LPO), and Acetilcolinestarase (Ache).

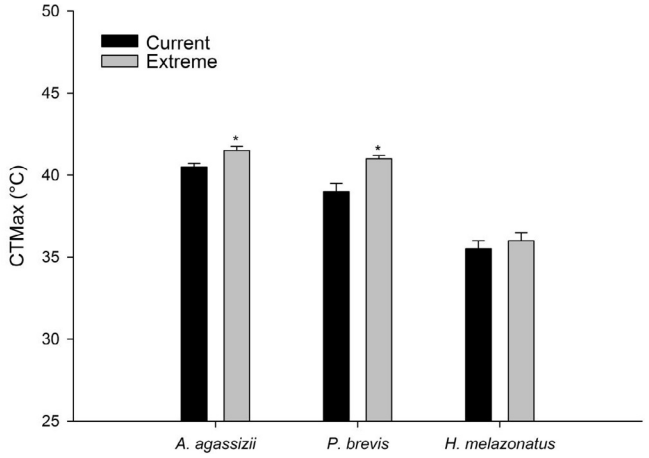

Fig. 5. Critical thermal maximum of three Amazon forest stream fishes, *Appistogramma agassizii* (current scenario 40 °C ± 0.2 and extreme 41.5 °C ± 0.2), *Pyrrhullina brevis* (current 39 °C ± 0.4 and extreme 40 °C ± 0.2) and *Hyphessobrychon melazonatus (*current 36 °C ± 0.5 and extreme 36 °C ± 0.5) acclimated for 30 days at climate change scenarios (IPCC, 2014). * indicates significant different from current scenario using *t*-test ($p < 0.05$).

uncoupling mitochondrial function due to membrane cell disruption, with the potential occurrence of apoptosis and less ATP generation (Iftikar and Hickey, 2013). Our findings corroborate former oxidative stress studies with fish and crustaceos (Abele et al., 2002; Madeira et al., 2012; Sampaio et al., 2018).

Climate alterations and oxidative stresses are known to induce tissue damage with potential effects on muscle contraction ability and swimming performance (Rosa et al., 2016). In fact, all species presented AChE alterations in extreme climate scenarios. AChE is an essential enzyme in the cholinergic pathway that hydrolyses acetylcholine (ACh), regulating its levels in the synaptic cleft (Drever et al., 2011). *Apistogramma agassizzi* and *P. brevis* increased Ache activities, the activation of AChE activity leads to a rapid degradation of ACh, an important neurotransmitter associated with locomotion (Jordan et al., 2014), suggesting that high temperature and CO_2 promotes synapse dysfunction, affecting the function of the cholinergic system. The rapid degradation of ACh can be associated with anxiety-like behaviour since the hyperactivation of the cholinergic system, in the brain is directly associated to increased anxiety-like behavior, as observed by Green and Jutfelt (2014) and Dixson et al. (2014). Instead, AChE inhibition in the species *H. melazonatus* could lead to ACh accumulation in the nerve, resulting in the disruption of nervous activities by overstimulation (Sharbidre et al., 2011). The prolonged stimulation of the muscle fibers causes paralysis which ultimately results in death (Purves et al., 2008). This result corroborated our findings that *H. melazonatus* present electron transport chain impairment that leading to important energy impacts and compromising its swimming performance.

4.2. The acclimation ability sets thermal limits

Our results show that *A. agassizii* and *P. brevis* presented higher thermal tolerance compared to *H. melazonatus*. In accordance, our previous findings (Campos et al., 2018) showed that *H. melazonatus* present low thermal tolerance related to its active life style, are reflected in its lower thermal acclimation ability, compared to the other two species studied herein. Increasing maintenance costs reduce physiological tolerance, once high metabolic rates may increase critical oxygen tension and thermal limitation results from a decrease in systemic oxygen levels (hypoxemia), which consequently is determined by the animal ability to extract oxygen and to efficiently deliver it to tissues (Pörtner and Knust, 2007).

Furthermore, critical thermal tolerances were significantly affected by climate scenario in *A. agassizii* and *P. brevis,* but not in *H. melazonatus*. *Apistogramma agassizii* and *P. brevis* presented higher thermal limits at extreme climate change scenario indicating a degree of acclimation capacities, supporting IBR data (discussed below). Both species showed a significant CTMax acclimation ability: *A. agassizii* increased its CTMax by 1.5 °C and *P. brevis* by 1.0 °C, in comparison to the current scenario. Our results corroborate previous studies with Amazon fish showing an acclimation capacity in their CTMax temperatures ranging from 1.0 °C to 1.8 °C (Campos et al., 2017). Acclimation capacity may actually be a mechanism that allows species to be resistant to climate changes; here we showed that some Amazon forest stream fishes have abilities to increase thermal tolerance, but *H. melazonatus* did not change its thermal limits, suggesting that climate change will be specially a threat to this species.

4.3. Integrated biomarker response as a tool to identify acclimation capacity

Molecular mechanisms supporting thermal limits have been associated with functional acclimation capacity of energetic metabolism and antioxidant defense to maintain homeostasis (Pörtner and Knust 2007; Madeira et al., 2016a). Therefore, studying cellular and biochemical responses can be an important tool for defining the thermal window and thermal tolerance of a species (Deschaseaux et al., 2010). The use of a methodology that integrates the responses of different biomarkers has been used to identify early thermal stress that could be related to acclimation abilities. Among these indices, Madeira et al. (2016b) have satisfactorily applied the Integrated Biomarker Response (IBR) in distinct tissues (including white muscle). In that work, the authors suggested that acclimation capacities in *Amphiprion ocellaris* are time-dependent and IBR values stabilized after 30 days of exposure;

therefore, no significant differences between the control and elevated temperature could be related to acclimation capacities.

Our findings, however, suggest that the three analyzed species presented physiological stress in the present study, since they all increased IBR values at extreme climate scenario compared to the current one. However, *H. melazonatus*, the athletic species, had severe molecular alterations suggesting a narrow ability to tolerate further warming. The high increased IBR value results from oxidative stress, membrane damage, and loss of muscle neurotransmitter. Madeira et al. (2018) also observed an increase in IBR for distinct marine tropical species related to oxidative damage and induction of protective pathways against ROS damage. Therefore, the high mortalities and narrow thermal safety margin (the difference between thermal tolerance and thermal average) observed in the present study for *H. melazonatus* results from low acclimation abilities and cellular stress. All these data are corroborated by cluster analyses and heatmaps that show a climate scenario cluster, suggesting a strong influence of temperature and CO_2 under biochemical and physiological parameters.

Our data suggest that all Amazonian species herein studied have low acclimation capacities at climate change scenario after 30 days exposure (Figs. 3 and 4). As far as we know, this is the first study that has examined thermal acclimation abilities in Amazon forest stream fish, which are adapted to a more thermally stable environment. Moreover, for most of the studied tropical species, including Amazonian fishes, studies have indicated that they have limited capacity for warming acclimation (Stillman, 2003; Campos et al., 2018). For instance, two congeneric species, *Paracheirodon axelrodi* and *P. simulans,* exposed for 28 days at 32 °C did not improve their aerobic capacity compared to natural temperature (Campos et al., 2017). Indeed, for these same species acclimated to A2 climate change scenario (4.5 °C and 900 ppm above current scenario), Fé-Gonçalves et al. (2018) found limited activation of glycolytic metabolism in *P. axelrodi*, which was related to increased mortalities. In conformity, Madeira et al (2018), applying IBR methodology in four tropical species from distinct Phyla (Fishes, Mollusca and Crustacea), suggested low acclimation abilities for 3 °C warming, with all species presenting high IBR index values.

As for marine species, temperate streams species have high acclimation capacities compared to tropical ones. Rooke et al. (2017) showed that cold population of sunfish (*Lepomis gibbosus*) present high energetic and thermal plasticity compared to warm populations. Therefore, our results corroborate that tropical species are especially in threat under climate change scenario.

Our results suggest that Amazon forest streams fishes will be strongly affected by climate change scenario due to their low acclimation ability. Acclimation is the result of cellular and biochemical responses to maintain physiological homeostasis, which includes energy supply, and avoid cellular stress (Madeira et al., 2016b). In fact, the decrease in ROS formation, protein and lipid damage in fishes acclimated to warming has been related to their capacity to maintain thermal performance and improve their tolerance (Sampaio et al., 2018). Besides, our results confirm that IBR index is sensitive, provides precise information about species acclimation capacity, and should be used as a tool to evaluate the early effects of climate change on physiological stress as suggested by Madeira et al., 2018.

5. Conclusions

Overall, the Amazon species studied herein are susceptible to extreme climate change and stream warming caused by deforestation since them all present metabolic and oxidative alterations. The sedentary ones *A. agassizii* and *P. brevis* seem to be less vulnerable to climate change because they present low levels of mortalities, reduced metabolic alterations and high thermal tolerance under a extreme warming scenario. In contrast, *H. melazonatus,* the athletic species, presents the most severe damage and alterations, which is related to high mortality levels and narrow thermal safety margin.

These results corroborate our previous findings for fish acclimatized to stable thermal forest habitats, i.e., that athletic species are more vulnerable to warming (Campos et al., 2018), indicating that future climate scenario will have lower impact on low swimming performance species with potential effects on ecosystem metabolism.

Based in our findings, we suggest that *H. melazonatus,* and possibly other athletic species, might be used for field monitoring studies applying the IBR methodology of aerobic metabolism and antioxidant defense enzymes to verify early effects of climate change. These studies provides important evidences for the impacts of climate change on population of ornamental fishes. In addition, future climate scenario and ornamental trade can both impact the genetic variability of Amazon species, which can be critical in species that are especially vulnerable as high activity species like *H. melazonatus*.

Acknowledgments

The authors would like to thank the Brazilian National Research Council (CNPq) and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) to support the INCT ADAPTA (CNPQ process N° 465540/2014-7 and FAPEAM process 062.01187/2017). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. VV and ALV are recipients of Research fellowships from CNPq and DFC and SBM are recipients of doctoral fellowships from CAPES.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.01.051.

References

Abele, D., Heise, K., Pörtner, H.O., Puntarulo, S., 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J. Exp. Biol. 205, 1831–1841.

Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21, 1316–1322.

Beitinger, T.L., Bennett, W.A., 2000. Quantification of the role of acclimation temperature in temperature tolerance of fishes. Environ. Biol. Fish. 58, 277–288.

Beutler, E., 1975. In: Red Cell Metabolism: A Manual of Biochemical Methods. Grune & Straton, New York, pp. 678.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Campos, D.F., Jesus, T.F., Kochhann, D., Heinrichs-Caldas, W., Coelho, M.M., Almeida-Val, V.M.F., 2017. Metabolic rate and thermal tolerance in two congeneric Amazon fishes: *Paracheirodon axelrodi* Schultz, 1956 and *Paracheirodon simulans* Géry, 1963(Characidae). Hydrobiology 789, 133–142.

Campos, D.F., Val, A.L., Almeida-Val, V.M.F., 2018. The influence of lifestyle and swimming behavior on metabolic rate and thermal tolerance of twelve Amazon forest stream fish species. J. Ther. Biol. 72, 148–154.

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D.S., Xia, J., 2018. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucl. Acids Res. https://doi.org/10.1093/nar/gky310.

Dean, J.B., 2010. Hypercapnia causes cellular oxidation and nitrosation in addition to acidosis: implications for CO_2 chemoreceptor function and dysfunction. J. Appl Physiol. 108, 1786–1795.

Deschaseaux, E.S.M., Taylor, A.M., Maher, W.A., Davis, A.R., 2010. Cellular responses of encapsulated gastropod embryos to multiple stressors associated with climate change. J. Exp. Mar. Bio. Ecol. 383 (130–136), 2009. https://doi.org/10.1016/j.jembe.2009.12.013.

Dixson, D.L., Jennings, A.R., Atema, J., Munday, P.L., 2014. Odour tracking in sharks is reduced under future ocean acidification conditions. Global Change Biol. https://doi.org/10.1111/gcb.12678.

Drever, B.D., Riedel, G., Platt, B., 2011. The cholinergic system and hippocampal plasticity. Behav. Brain Res. 221, 505–514.

Driedzic, W.R., Almeida-Val, V.M.F., 1996. Enzymes of cardiac energy metabolism in Amazonian teleosts and the Hydrobiologia 123 fresh-water stingray (*Potamotrygon hystrix*). J. Exp. Zool. 274, 327–333.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282.

Ellman, G.L., Courtney, K.D., Andres, J.V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7,

88–95.
Farrell, A.P., 2007. Cardiorespiratory performance during prolonged swimming tests with salmonids: a perspective on temperature effects and potential analytical pitfalls. Philos. Trans. R. Soc. Lond. B Biol. Sci. 362, 2017–2030.
Fé-Gonçalves, L.M., Paula-Silva, M.N., Val, A.L., Almeida-Val, V.M.F., 2018. Differential survivorship of congeneric ornamental fishes under forecasted climate changes are related to anaerobic potential. Gen. Mol. Biol. https://doi.org/10.1590/1678-4685-GMB-2017-0016.
Ganachaud, A.S., Gupta, A.S., Orr, J.C., Wijffles, S.E., Ridgway, K.R., Hemer, M.A., Maes, C., Steinberg, C.R., Tribollet, A.D., Qiu, B., 2011. Observed and expected changes to the tropical pacific ocean. In: Bell, J.D., Hobday, A.J. (Eds.), Vulnerability of Tropical Pacific Fisheries and Aquaculture to Climate Change. Secretariat of the Pacific Community, Noumea, pp. 101–187.
Green, L., Jutfelt, F., 2014. Elevated carbon dioxide alters the plasma composition and behaviour of a shark. Biol. Lett. 10, 2014.
G.-Tóth, L., 1993. Electron transport system (ETS) activity of the plankton, sediment and biofilm in Lake Balaton (Hungary). Verh. Int. Ver. Limnol. 25, 680–681.
Iftikar, F.I., Hickey, A.J.R., 2013. Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in *Notolabrus celidotus*. PLoS One 8, e64120.
Ilha, P., Schiesari, L., Yanagawa, F.I., Jankowski, K., Navas, C.A., 2018. Deforestation and stream warming affect body size of Amazonian fishes. PLoS One 13 (5), e0196560. https://doi.org/10.1371/journal.pone.0196560.
IPCC, 2014. Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. In: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B. (Eds.), Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1132.
Hofmann, G., Somero, G., 1995. Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel *Mytilus trossulus*. J. Exp. Biol. 198, 1509–1518.
Jiang, Z.Y., Woollard, A.C.S., Wolff, S.P., 1991. Lipid hydroperoxide measurement by oxidation of Fe^{2+} in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids 26 (10), 853–856. https://doi.org/10.1007/BF02536169.
Jesus, T.F., Moreno, J.M., Repolho, T.A., Rosa, R., Almeida-Val, V.M.F., et al., 2017. Protein analysis and gene expression indicate differential vulnerability of Iberian fish species under a climate change scenario. PLoS One 12 (7), e0181325.
Jordan, L.M., McVagh, J.R., Noga, B.R., Cabaj, A.M., Majczynski, H., Slawinska, U., Provencher, J., Leblond, H., Rossignol, S., 2014. Cholinergic mechanisms in spinal locomotion – potential target for rehabilitation approaches. Front. Neural Circ. 8, e132.
Kenner, R.A., Ahmed, S.I., 1975. Measurements of electron transport activities in marine phytoplankton. Mar. Biol. 33, 119–127.
Keen, J.H., Habig, W.H., Jakoby, W.B., 1976. Mechanism for several activities of the glutathione-S-transferase. J. Biol. Chem. 251 (20), 6183–6188.
Lopes, A.R., Sampaio, E., Santos, C., Couto, A., Pegado, M.R., Diniz, M., Munday, P.L., Rummer, J.L., Rosa, R., 2018. Absence of cellular damage in tropical newly hatched sharks (*Chiloscyllium plagiosum*) under ocean acidification conditions. Cell Stress Chaperon. 23 (5), 837–846. https://doi.org/10.1007/s12192-018-0892-3.
Lough, J., 2007. Climate and climate change on the Great Barrier Reef. In: Johnson, J.E., Marshall, P.A. (Eds.), Climate Change and the Great Barrier Reef. Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia, pp. 14–50.
Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and critique. Can. J. Zool. 75, 1561–1574.
Macedo, M.N., Coe, M.T., DeFries, R., Uriarte, M., Brando, P.M., Neill, C., Walker, W.S., 2013. Land-use-driven stream warming in southeastern Amazonia. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20120153. https://doi.org/10.1098/rstb.2012.0153.
Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., Diniz, M.S., 2012. HSP70 production patterns in coastal and estuarine organisms facing increasing temperatures. J. Sea Res. 73, 137–147.
Madeira, D., Vinagre, C., Diniz, M.S., 2016a. Are fish in hot water? Effects of warming on oxidative stress metabolism in the commercial species *Sparus aurata*. Ecol. Indic. 63, 324–331.
Madeira, C., Madeira, D., Diniz, M.S., Cabral, H.N., Vinagre, C., 2016b. Thermal acclimation in clownfish: an integrated biomarker response and multi-tissue experimental approach. Ecol. Indic. 71, 280–292. https://doi.org/10.1016/j.ecolind.2016.07.009.
Madeira, C., Mendonça, V., Leal, M.C., Flores, A.A.V., Cabral, H.N., Diniz, M., Vinagre, C., 2018. Environmental health assessment of warming coastal ecosystems in the tropics – application of integrative physiological indices. Sci. Total Environ. 643, 28–39.
Marengo, J.A., 2009. Future change of climate in South America in the late 21st century: the CREAS project. Atmos. Sci. Sect. AGU Newsl. 3 (2), 5.
Meehl, G.A., Stocker, T.F., Collins, W.D., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., 2007. Global climate projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis. Cambridge University Press, Cambridge and New York, pp. 747–784.
Mendonça, F.P., Magnusson, W.E., Zuanon, J., 2005. Relationships between habitat characteristics and fish assemblages in small streams of central Amazonia. Copeia 2005, 751–764.
Packard, T.T., 1971. The measurement of respiratory electron-transport activity in marine phytoplankton. J. Mar. Res. 29, 235–244.
Pannunzio, T.M., Storey, K.B., 1998. Antioxidant defenses and lipid peroxidation during anoxia stress and aerobic recovery in the marine gastropod *Littorina littorea*. J. Exp. Mar. Biol. Ecol. 221, 277–292.
PBMC, 2013. In: Contribuição do Grupo de Trabalho 1 ao Primeiro Relatório de Avaliação Nacional do Painel Brasileiro de Mudanças Climáticas. Sumário Executivo GT1. PBMC, Rio de Janeiro, Brasil, pp. 24.
Peck, M.A., Huebert, K.B., Llopiz, J.K., 2012. Intrinsic and extrinsic factors driving match-mismatch dynamics during the early life history of marine fishes. Adv. Ecol. Res. 47, 177–302.
Pörtner, H.O., Farrell, A.P., 2008. Physiology and climate change. Science 322 (5902), 690–692. https://doi.org/10.1126/science.1163156.
Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315, 95–97.
Pörtner, H.O., Schulte, P.M., Wood, C.M., Schiemer, F., 2010. Niche dimensions and limits in fishes: an integrative view. Illustrating the role of physiology in understanding ecological realities. Physiol. Biochem. Zool. 83 (5), 808–826. https://doi.org/10.1086/655977.
Pörtner, H.O., Karl, D.M., Boyd, P.W., Cheung, W.W.L., Lluch-Cota, S.E., Nojiri, Y., Schmidt, D.N., Zavialov, P.O., 2014. Ocean systems. In: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), Climate Change 2014: Impacts, Adaptation, and Vulnerability Part A: Global And Sectoral Aspects Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 411–484.
Purves, D., George, J.A., David, F., William, C.H., Anthony Samuel, L., James, O.M., Leonard, E.W., 2008. ISBN-13: 978-0878936977 In: Neuroscience, fourth ed. Sinauer Associates, Massachusetts, USA, pp. 857.
Rooke, A.C., Burness, G., Fox, M.G., 2017. Thermal physiology of native cool-climate, and non-native warm-climate Pumpkinseed sunfish raised in a common environment. J. Therm. Biol. 64, 48–57. https://doi.org/10.1016/j.jtherbio.2016.12.010.
Rosa, R., Paula, J.R., Sampaio, E., Pimentel, M., Lopes, A.R., Baptista, M., Guerreiro, M., Santos, C., Campos, D., Almeida-Val, V.M.F., Calado, R., Diniz, M., Repolho, T., 2016. Neuro-oxidative damage and aerobic potential loss of sharks under elevated CO2 and warming. Mar. Biol. 163, (5). https://doi.org/10.1007/s00227-016-2898-7.
Sampaio, E., Lopes, A.R., Francisco, S., Paula, J.R., Pimentel, M., Maulvault, A.L., Repolho, T., Grilo, T.F., Pousão-Ferreira, P., Marques, A., Rosa, R., 2018. Ocean acidification dampens physiological stress response towarming and contamination in a commercially-important fish(*Argyrosomus regius*). Sci Total Environ. 618, 388–398.
Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol. 213, 912–920.
Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science 301, 65.
Schulte, P.M., 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. J. Exp. Biol. 218 (12), 1856–1866.
Semsar-kazerouni, M., Verberk, W.C.E.P., 2018. It's about time: Linkages between heat tolerance, thermal acclimation and metabolic rate at different temporal scales in the freshwater amphipod *Gammarus fossarum* Koch, 1836. Ther. Biol. https://doi.org/10.1016/j.jtherbio.2018.04.016.
Sharbidre, A.A., Metkari, V., Patode, P., 2011. Effect of diazinon on acetylcholinesterase activity and lipid peroxidation of *Poecilia reticulata*. Res. J. Environ. Toxicol. 5, 152–161.
Simčič, T., Brancelj, A., 2003. Estimation of the proportion of metabolically active mass in the amphipod *Gammarus fossarum*. Freshwater Biol. 48, 1093–1099.
Stoffels, R.J., 2015. Physiological trade-offs along a fast-slow lifestyle continuum in fishes: what do they tell us about resistance and resilience to hypoxia? PLoS One 10 (6), e0130303 (pmid:26070078).
Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Putting the heat on tropical animals. Science 320, 1296–1297.
Tomanek, L., 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. J. Exp. Biol. 213, 971–979.
Vinagre, C., Madeira, D., Narciso, L., Cabral, H., Diniz, M., 2012. Impact of climate change on coastal versus estuarine nursery areas: cellular and whole-animal indicators in juvenile seabass, *Dicentrarchus labrax*. Mar. Ecol. 464, 237–243.
Wu, R.S.S., Siu, W.H.L., Shin, P.K.S., 2005. Induction, adaptation and recovery of biological responses: implications on environmental monitoring. Mar. Pollut. Bull. 51, 623–634.